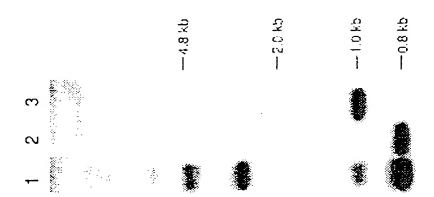


FIGURE 1



λ E7

Bam H1 fragments

E7.3 Eco R1 fragments E7.14

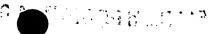
E1.5

1 kb

E7.31

E7.8

E7.18



RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	1 meinfkvlsk	.*******p**	pkv*sgas*n prp*a*	***h***aa*	pg******* **s* *lkf*sqers ******ggk
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	51 1**v* 1**l**qc  wd*s*t*k rlsv*p***f SV-SVP-	*rv*kde*mk	***sf*s***	<pre>q*d*****ak prdy****a* d*ks**psv* d**s***pl* rg**ia**</pre>	
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	101 ********  *******  ******  lnv*ss**p* ln***t**p*  ****1**ae*  YDLDPKLE-F	********* ************ *********** 1********	*****gs**e *****s***  **h**k***e *v***m****	**********  n**s**s***  *********  y****q**a*  y**p****ag  ***s*****  HEGGLEEFSK	150 *********  *******  ******  *****  GYLKFGINTE
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	151 *g****** *dg***** nd***** *dgis**** *gci**** hg*s*****ATVYREWA	******** ********* ******** ********	*********** ***d***a** ***g*****1 ***g****** ***g****** ***g******	******** ******** r*t**n*** h****q*** m****q*** **a**n*** KMEKD-FGVW	200 **k*****  **k*d**k**  **q*pdad*n  ****pd*ds*  ********  SIRISHVNGK
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	********** **************************	***k*sd*** ***k**n***	**************************************	********* **f******* ****ptr*a* **a**t**a* ***t**es**	********* ********** ***********
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	1****q**** p****h**y* s*****n**	**S**a*** **r****** *****k***	************ ************************	k*a******  r*******  **r*ns****  kl*ag****	********** **d******* **d*********** p*****cl**

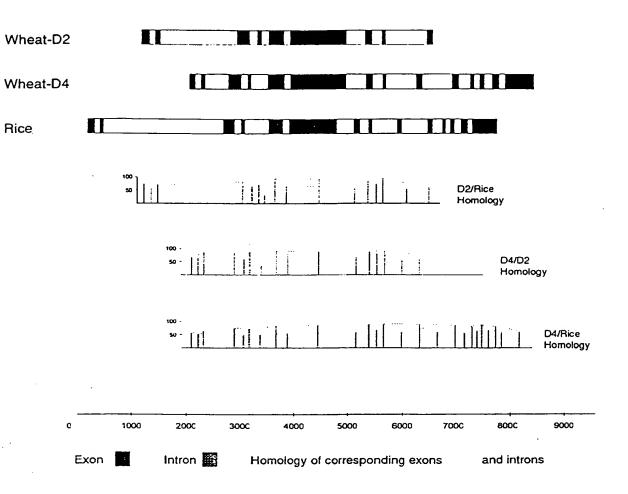
DOEDBY'' LEGDED

RSBEI	301	*****	*****	*****	350
MSBEI	******			******	
D4cDNA	******	*****ilcf*	w*****.**	*******	*******
PESBEII	*******	*****		******S***	*******
POSBE	*****	******g**	******	****y*n***	*****
D2cDNA	t*******	*****ds***	*****.**	******	******
Consensus	NNYNTVQLMA	IMEHSYYASF	GYHVTN-FFA	VSSRSGTPED	LKYL-DKAHS
	351				400
RSBEI		*****	********	*h*****	******
MSBEI	******	******	*****	******a**	******
D4cDNA	******	*****s*m**	********	******	******
PESBEII	***n*****	******	******	s*q****a**	*****
POSBE	***q**v***	******	********	s*****a**	*******
D2cDNA	******	********i*	*****	ah****yt**	k**n***ng*
Consensus	LGLRVLMDVV	HSHASNNVTD	GLNGYDVGQS	TQESYFH-GD	RGYHKLWDSR
	401				450
RSBEI	******	*****	******	*****	
MSBEI	*****	*****		*****	
D4cDNA	*****	*****		`*******n	
PESBEII	******ks.	S******		******	
POSBE	******	******		********V	
D2cDNA	******	*****		*v******n	
Consensus	LFNYANWEVL	RFLLSNLRYW	-DEFMEDGER	FDGVTSMLYH	HHGTNMGFTG
			DEI III DGI K	I DOVI DIADA	
		gam"	bbi Mi bgi K	1001101111	
	451	jar.			500
RSBEI MSBEI	451	*****	******1**	*****	500
RSBEI MSBEI	451 ********	*************	******1** ******1**	*****	500 *********
RSBEI	451 ******* **q****** *****g***	*********	******1** ******1** *******1**	******** *****	500
RSBEI MSBEI D4cDNA	451 ******* **q***** *****g*** d*n****e**	******** ****************************	******1** ******1**	********* *********	500 *******  ******  ******  ***g*g***s
RSBEI MSBEI D4cDNA PESBEII	451 *******  **q******  ******g*** d*n****e**  **n****ea*	*********	*******1** ******1** ******i** **s*v*di**	******** *****	500 ******* ********
RSBEI MSBEI D4cDNA PESBEII POSBE	451 *******  **q******  ******g*** d*n****e**  **n***ea*  *****ig***	********* ***************************	*******1** ******1** ********* **s*v*di** **n*i**i**	********* *********	500 *******  ******  ***g*g***  ***g*g***  ***g*g***
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT	******** a******* ******* ********	*******1** ******1** *******1** **s*v*di** **n*i**i** ******1**	******** ******** ********* ********	500 *******  ******  *****  ***g*g***  ***g*g***  CMPVLCRPVD
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	451 ******** **q***** d*n***e* **n***ea* ****ig*** NYKEYFSLDT	******** a******* ******** ******** n***f***** DVDAVVYMML	*******1** ********  **s*v*di** **n*i**i** ******1** ANHLMHK-LP	******** ******** ***d***** ******** **i***v*** EATVVAEDVS	500 ******* ****** ***** ***g*g*** ***g*g*** GMPVLCRPVD
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA	451 ******** **q****** d*n****e** **n***ea* ****ig*** NYKEYFSLDT  501 ********	*********  ********  *******  DVDAVVYMML  *******	*********  *******  *******  *******  ANHLMHK-LP  *********	*********  *******  *****  ****  EATVVAEDVS	500 ********  *******  ***g*g***  ***g*g***  ******
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	451 ******** **q***** d*n***e* **n***ea* ****ig*** NYKEYFSLDT	*********  ********  *******  DVDAVVYMML  *******	**********  *******  *******  ********	*********  *******  *****  ****  EATVVAEDVS  ****.*vq**  **g*.*ah**	500 ******* ****** ***** ***g*g*** ***g*g*** GMPVLCRPVD
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	451 ******** **q****** d*n****e** **n***ea* *****ig*** NYKEYFSLDT  501 *********	*********  ********  *******  n***f****  DVDAVVYMML  ********	**********  *******  *******  *******  ANHLMHK-LP   ********  ************************	*********  *******  *****  ****  EATVVAEDVS	500 ********  *******  ***g*g***  ***g*g***  ***g*g***  GMPVLCRPVD  550  **********  *********
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA	451 ******** **q****** d*n****e** **n***ea* *****ig*** NYKEYFSLDT  501 ***********	*********  ********  *******  n***f****  DVDAVVYMML  ********  *********	**********  *******  *******  ********	*********  ********  *******  *******  EATVVAEDVS  ****.*vq**  **g*.*ah**  ***a.*ah**	500 ********  *******  *******  ***g*g***  ***g*g***  ******
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA PESBEII	451 ******** **q****** d*n***e** **n***ea* ****ig** NYKEYFSLDT  501 ******** ********** **********	*********  a*******  ********  n***f****  DVDAVVYMML   *******  *******  ********  ********	**********  *******  *******  *******  ANHLMHK-LP   ********  ********  **********  *****	*********  ********  *******  **i***v**  EATVVAEDVS   ****.*vq**  **g*.*ah**  ***a.*ah**  **k*.*sln*	500 ******* ****** ****** ***** *****  ****
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA PESBEII POSBE	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT  501 ******** ******** ******** ********* ****	*********  a*******  ********  n***f****  DVDAVVYMML   *******  *******  ********  ********	*******!**  *******!**  *******!**  ANHLMHK-LP   *********  ********  *********  *****	********  *******  *****  ****  ****  ****	500 ******** ******* ****** ****** ******
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT  501 ******** ******** ******** ********* ****	*********  a*******  ********  n***f****  DVDAVVYMML   ********  ********  ********  ******	*******!**  *******!**  *******!**  ANHLMHK-LP   *********  ********  ********  ******	********  *******  *****  ****  ****  ****	500 *******  ******  ******  *****  ****  ****
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT  501 ******** ******* ******* ******** *****	*********  a*******  ********  n***f****  DVDAVVYMML   ********  ********  ********  ******	*******!**  *******!**  *******!**  ANHLMHK-LP   *********  ********  ********  ******	********  *******  ******  *****  ****  ****	500 *******  ******  ******  ***g*g***  ***g*g***  ******
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT  501 ******** ******* ******* ******** *****	*********  ********  ********  n***f****  DVDAVVYMML   *******  *******  *******  *******  AMAIPDRWID	*********  *******  ******  ******  ****	********  *******  *****  ****  ****  ****	500 *******  ******  ******  *****  ****  ****
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus  RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT  501 ******** ******* ******* ******* ******	*********  ********  ********  n***f****  DVDAVVYMML   *******  *******  *******  AMAIPDRWID	*******!**  *******!**  *******!**  ANHLMHK-LP   ********  *******  *******  *******  ****	********  *******  *******  ******  ****	500 ********  *******  ******  ******  ****
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT  501 ******** ******* ******* ******* ******	*********  ********  *******  n***f****  DVDAVVYMML   *******  *******  *******  AMAIPDRWID  ********  **********	*********  *******  *******  *******  ANHLMHK-LP   *******  ******  ******  ******  ****	********  *******  ******  *****  ****  ****	500 ******* ****** ****** ***** ***** *****
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI MSBEI D4cDNA PESBEII POSBE	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT  501 ******** ******* ******* ******* ******	*********  ********  ********  n***f****  DVDAVVYMML   ********  ********  ********  AMAIPDRWID  ********  *********  ***********  ****	*********  *******  *******  *******  ANHLMHK-LP   *******  ******  ******  ******  ****	********  *******  ******  *****  ****  ****	500 *******  *******  ******  ******  *****
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus RSBEI MSBEI MSBEI D4cDNA PESBEII	451 ******** **q****** d*n***e** **n***ea* ****ig*** NYKEYFSLDT  501 ******** ******* ******* ******* ******	*********  ********  ********  ********	*******!**  ******!**  ******!**  ******	********  *******  ******  *****  ****  ****	500 *******  *******  ******  ******  *****

Figure 4 (cont..)

design of He

RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	********* ********** *f*******	**************************************	********	*********** **g****** ********	650 .******* .****** .***** lt**n*** .***n*a*s*RQWSLVDTD
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	********* ***************************	**********  *******  *r*** ****  *r***s***  v**vdtps**  DQAMNALD-K	********* ********* **i*a*t*** ****a*g*** C******n*t	********* ********* **st*n*** **s*d**n** a*h******g VSDMNEE-KV	******
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	701 ******** ****** ****** ***** *****  **thlrsgc* VFNFHP-KTY	k******** ********* ********* *ps** EGYKVGCDLP	******** ******** ******** ******** stssc** GKYRVALDSD	**V******  **V******  **m******  *te******  *we*****t  .*gpsnqspf AL-FGGHGRV	750 ********  aqyn***** ***a*q*** *******  skpfig*pgc GHDVDHFTSP
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	******** ******** ifcc*lfkge	*****  ****  **g*qipskc  *	cllrehvwli	********* telmnacq*l	800 ********  *******  ****h***v* kitrq*f*vs LSPPRTCVAY
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	*****ag *****ka ******q *yqqp*sr*v	<pre>agr*lhak*e *kpkde*** **snnpnlg* trnlkirylq</pre>	s**i.vte** t***s**es* w**aa*g.** *ee**a*adt *sv**tna*qGKT-PA-YI	**k*s*  **e***vkda  **aripdvs* klkf**qtf*	assk ad**at**sk e*ed*nld v*yyqapilr
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	edk*atagg* ka*tgg*ss* r*e*ns**av r*tr*lk*sl	**mk***r**  **wk*arqp*  **in***g*p dagi*kvere stnist*  KGFVF-SS	*q*t** *k*n*. vvgdn*		~



· 州北南洋

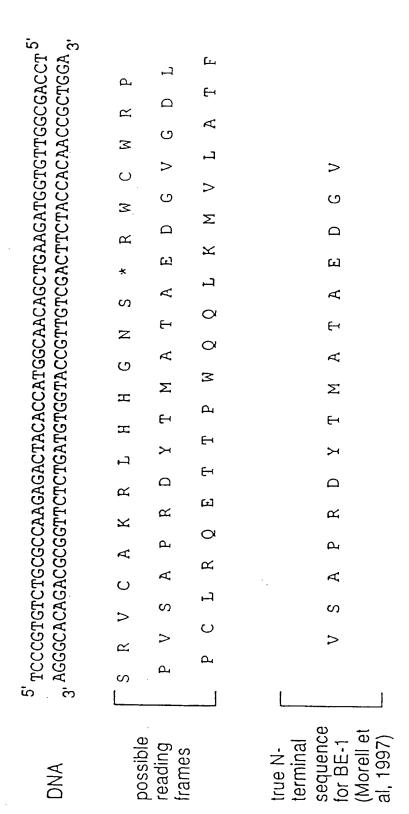


Figure 6



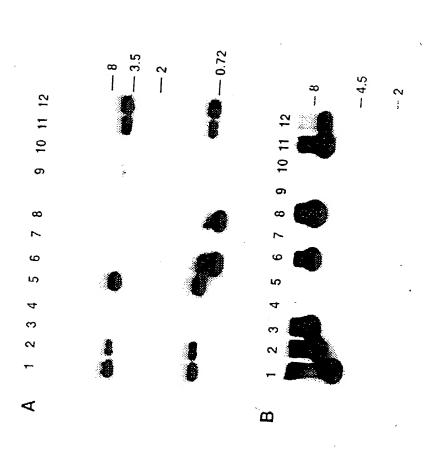


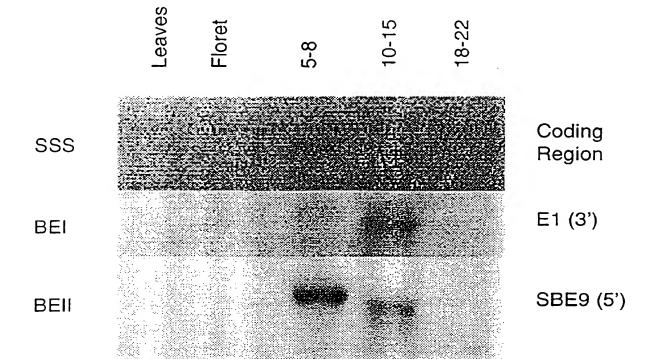
FIGURE 7



FIGURE 8



#### Expression of Starch Biosynthetic Genes



2.7 kb

#### 12/44

#### 4 6 8 10 12 15 18 21 25 31

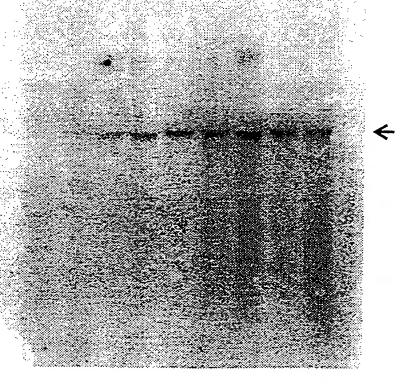
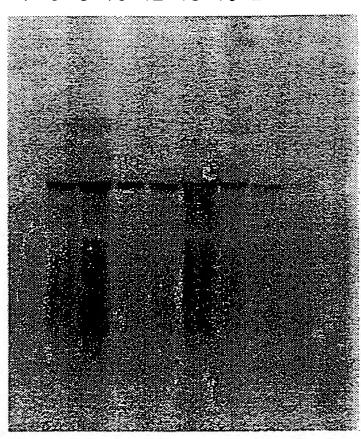


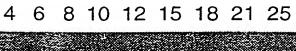
FIGURE 9B

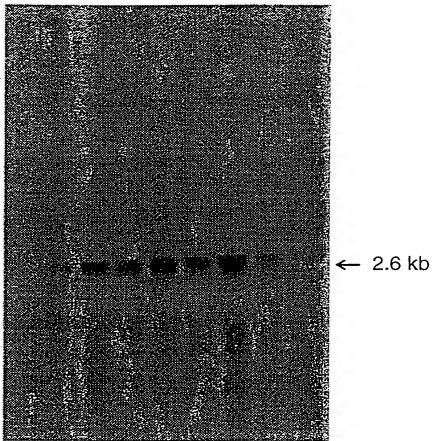
# 13/44

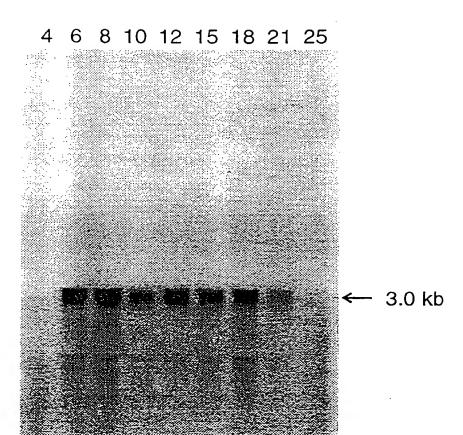
4 6 8 10 12 15 18 21 25 31



← 2.9 kb







ODEOWSy. TEloud

4 6 8 10 12 15 18 21 25

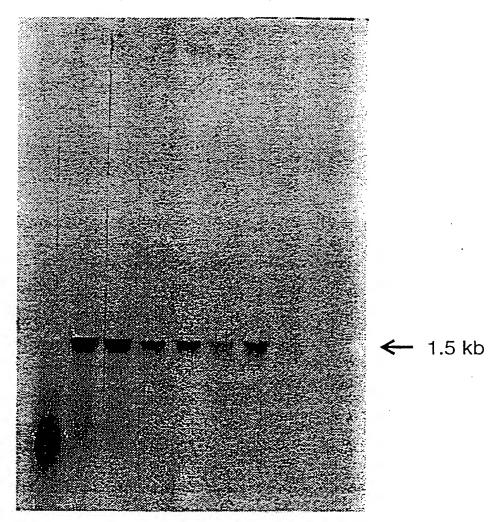
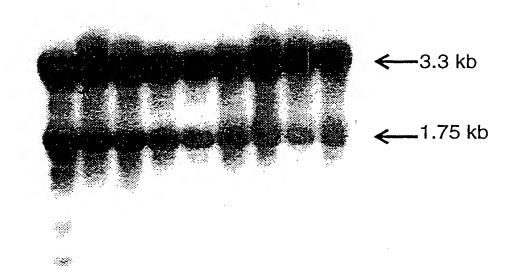
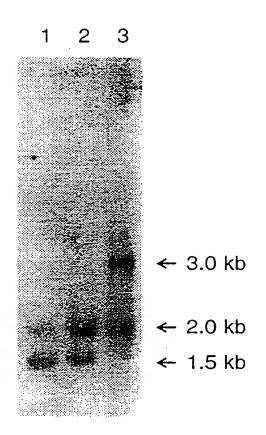


FIGURE 9F

4 6 8 10 12 15 18 21 25





DOTPLOT of: d10838.pnt Density: 12614.77 February 18, 1997 11:43

COMPARE Window: 21 Stringency: 14.0 Points: 20,788

sr427.res ck: 6,362, 1 to 11,099

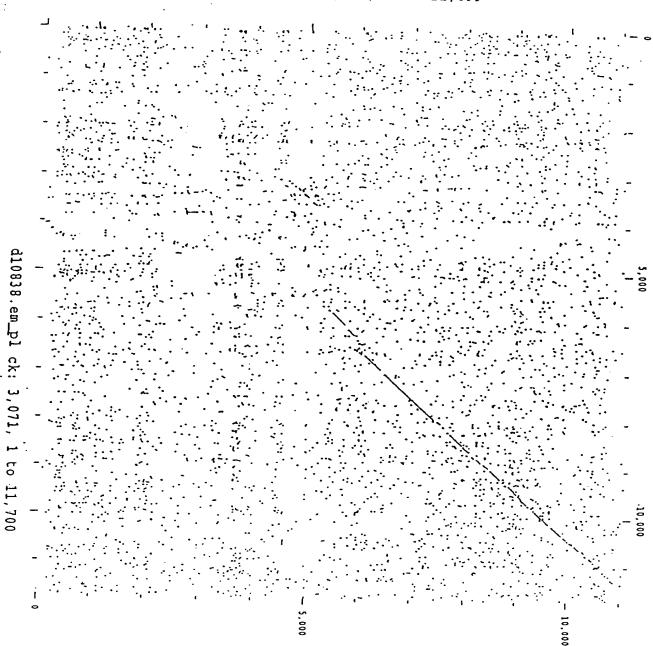


Figure 10

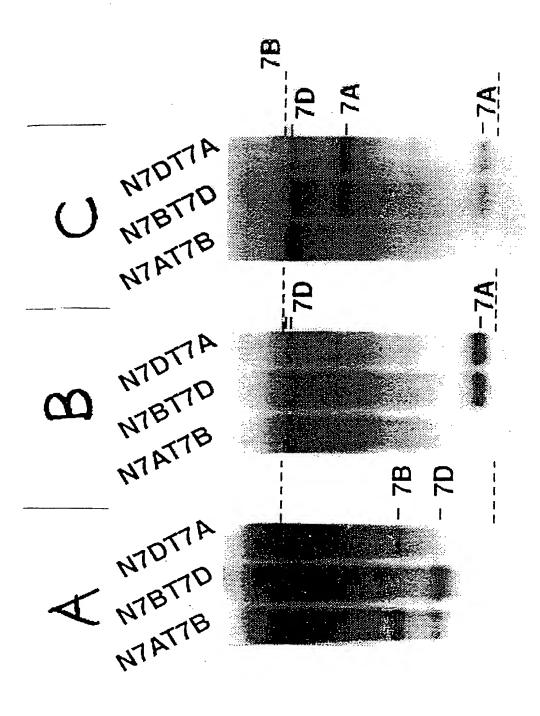


FIGURE 11

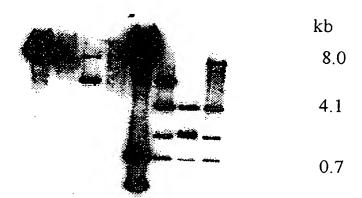
# 910 0 p (4310) 9 100 A 416

#### 21/44

# Genomic Clones from *T.tauschii* for SBE II.

BamH I EcoRI

#### F4 F3 F1 F4 F3 F3 F1



N-terminal sequences of cereal starch branching enzymes

DOECESY LOEDED

S > > SS < < Σ \_ \_ 0 Ö <u>۵</u> ت 2 s z S Ω <u>ය</u> ස  $\subseteq$ 5 5 G ΩШ ப 4 4 ۵ > > Σ ∠ ∑ 4 <  $\Sigma \Sigma$ > > > . ∢  $\bowtie$ Ш **5** 20 Q G d A 民田 S <u>ч</u> О S K 4 4 **&** > Ö 4 4 LSL ⋖ ~ 4 4 RICEBEII RICEBEI® WBE-IAD MAIZE MAIZE Protein WBE-II BEIle BEIc

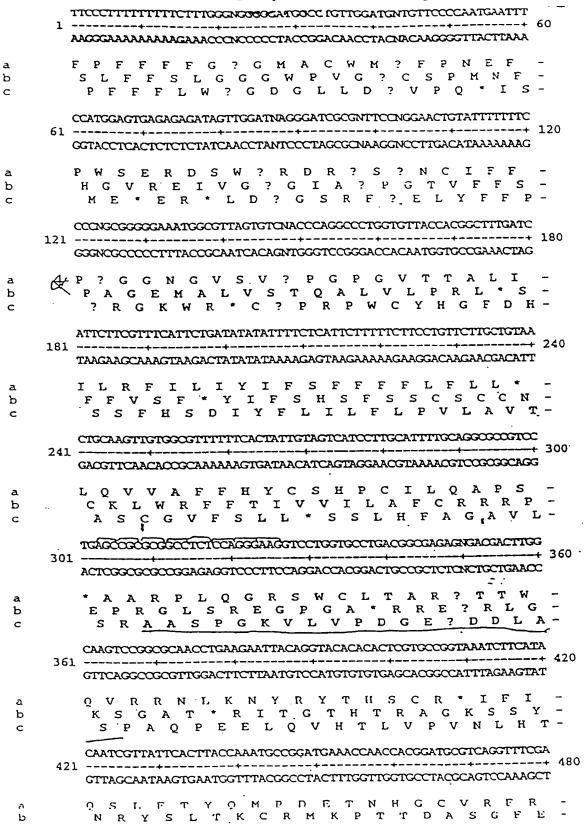
N-terminal amino acid of the mature polypeptide.
 Rawasaki et al.(1993), <sup>c</sup> Baba et al. (1991),

D Mizuno et al. (1993),8 Fisher et al. (1993)

Residues in the wheat sequences showing identity with the respective maize or rice branching enzyme isoforms are highlighted in bold text.

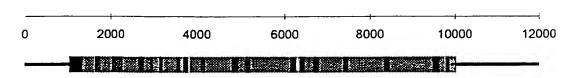
Figure 13a

b

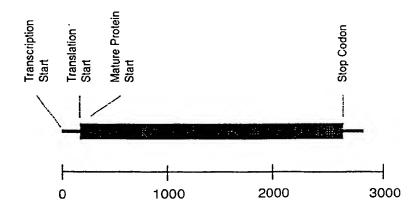


# Branching Enzyme-II Genes

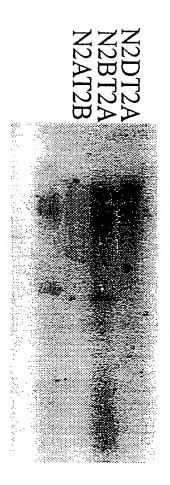
#### Intron/Exon structure of wheat BE-II



## Schematic Diagram of a cDNA for BE-II



Wheat DNA probed with the 5' conserved sequence of SBE II.



8kb

2kb

ossazz asoso

26/44

COMPARISON OF N-TERMINAL SEQUENCES OF SOLUBLE STARCH SYNTHASE

GRYVAELSREGPAARP

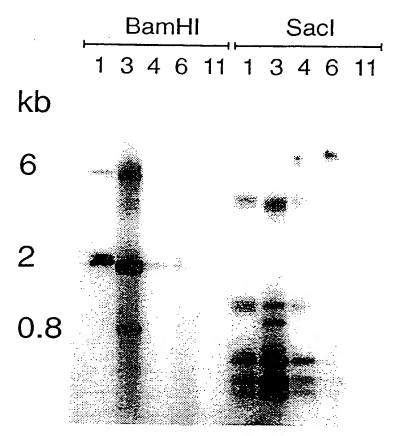
Deduced from wheat cDNA

Wheat N-terminal

GPYVAELSPEGPAAPP

Figure 16

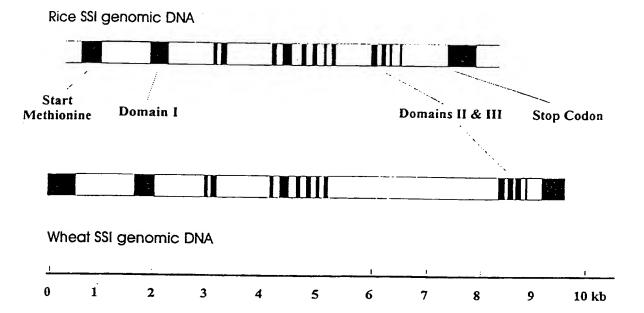
# Soluble Starch Synthase Genomic Clones



Probed with SM-2 full length cDNA



# **INTRON EXON STRUCTURE - Wheat SSI**



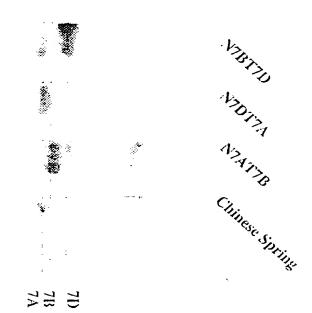


FIGURE 19

Enzymes that do not cut:

NONE

ECORI

```
139
                                                                                                                                        199
ATACTACATACTATGTGCTTGCACCCAAGGGACACTTTTATAACTATTCTGGCTGTGGGA
                         TATGATGTATGATATACGAACGTGGGTTCCCTGTGAAAATATTGATAAGACCGACACCCT
                                                                                                        ATACCTTCAACTGTAATCATCCTGTGGTTCGTCAATTCATTGTAGATTGTTAAGATACT
                                                                                                                                   TATGGAAGTTGACATTAGTAGGACACCAAGCAGTTAAGTAACATCTAACAATTCTATGA
                                                                                                                                                                                                                  GGGTGACGGAAATGCATGTTGATGGTTTTCGTTTTGACCTT
                                                                                                                                                                                                                                            CCCACTGCCTTTACGTACAACTACCAAAAGCAAAACTGGAA
                                                                                                                                                                                                                                                                                                     C
                                                                                                                                                                                                                                                                                         Σ
                                                                                                                                                                                                                                                                                                                         Enzymes that do cut
                                                                                                                                                                                                                                                                                                   Ö
                                                                                                                                                                                                                                                                                     G
             80
                                                                                                                       140
                                                                                                                                                                                                                               200
                                                      d
D
                                                                                                                                                               g Q O
                                                                                                                                                                                                                                                                        c Da
```

Figure 20a

Comparison of Wheat Debranching Fuzzm

Comparison of Wheat Debranching Enzyme-I (WDBE-I) PCR fragment with maize Sugary-1 DNA sequence	rcttcaat         rcttcaac	8 1167 1177 1187 1197 1207 1217 ATTATCCTTTAGGGGATAGTAATAGTACATACTTACATGCTTGCACCTAAGGGAAGATATATAT	.8         1227         1247         1257         1277           TTATAATTATTCTGGTTGTAATTCTAATTCTAATCCTGTGTAATT	8   1287   1397   1317   1337   1337   1337   1333   1334   1334   1333   133	8 1347 1357 dcrrgcarcraracr-g 	GE W B4% (219/260) INDOW 86% (219/253)
neat D	1098 TG	1158 TA      AT	1218 TT          	1278 TAT CAT	1338 dc [] CC	NG PERCENTAGE TOTAL WINDOW ALIGNMENT WINDOW
Comparison of w DNA sequence	SUGARY.DNA WHEATI.DNA	FILE NAME SUGARY.DNA WHEATI.DNA	FILE NAME SUGARY.DNA WHEATI.DNA	FILE NAME SUGARY, DNA WHEAT1, DNA	FILE NAME SUGARY.DNA WHEATI.DNA	MATCHING PERCENTAGE TOTAL WINDOW ALIGNMENT WIN

WO 99/14314

DOSDEZZ OSDOD

32/44

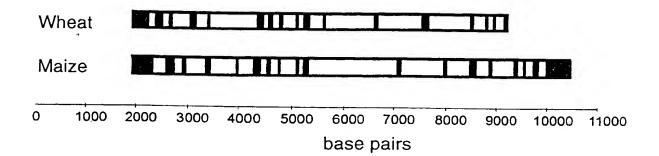
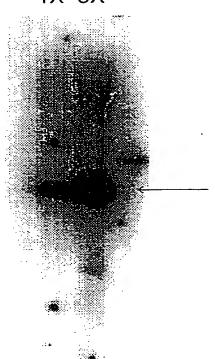


FIGURE 20C

## Southern blot of T. tauschii Genomic DNA





BamHI Digest

T. tauschii Genomic DNA Probed With The Wheat Debranching Enzyme PCR Product



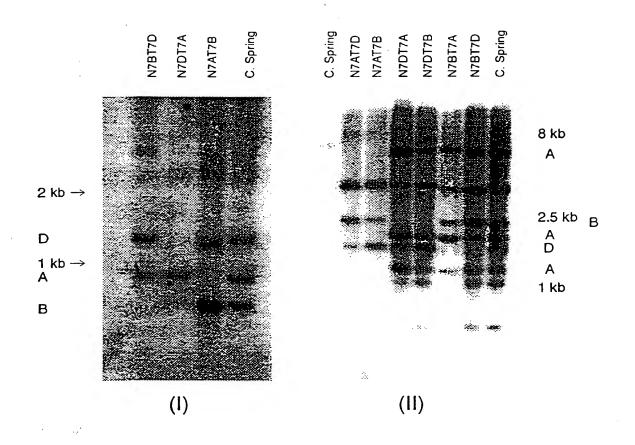


FIGURE 21B

35 / 44

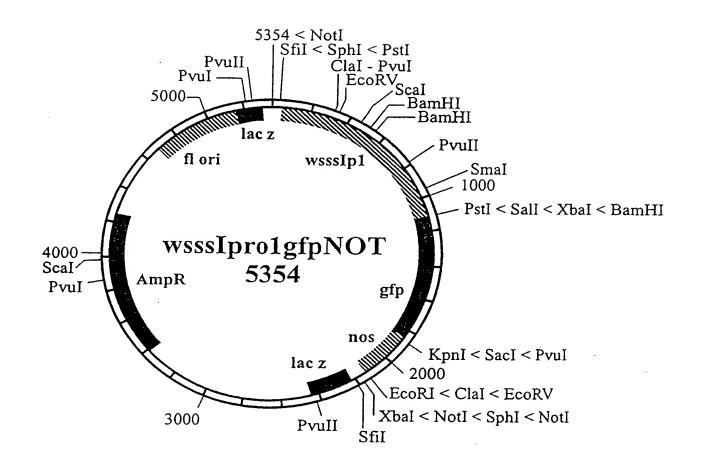


FIGURE 22A

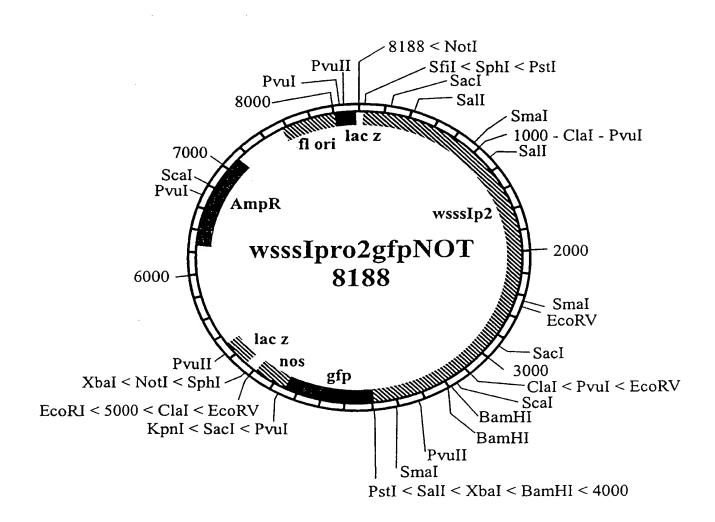


FIGURE 22B

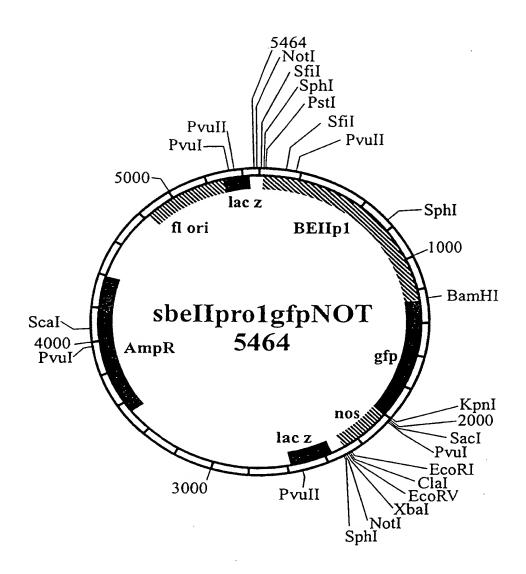
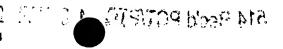


FIGURE 22C



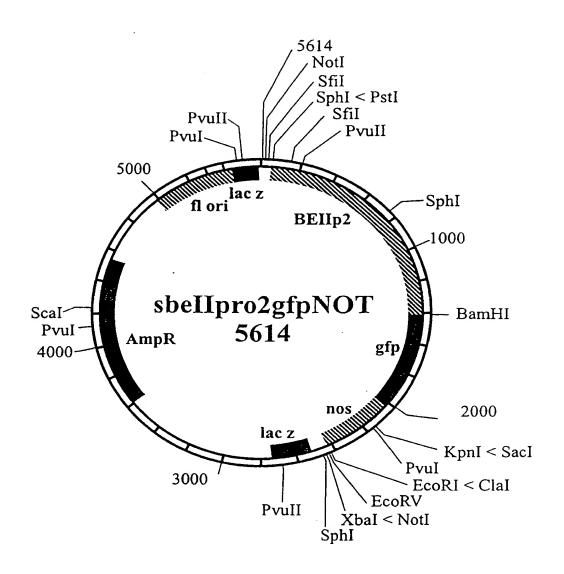


FIGURE 22D

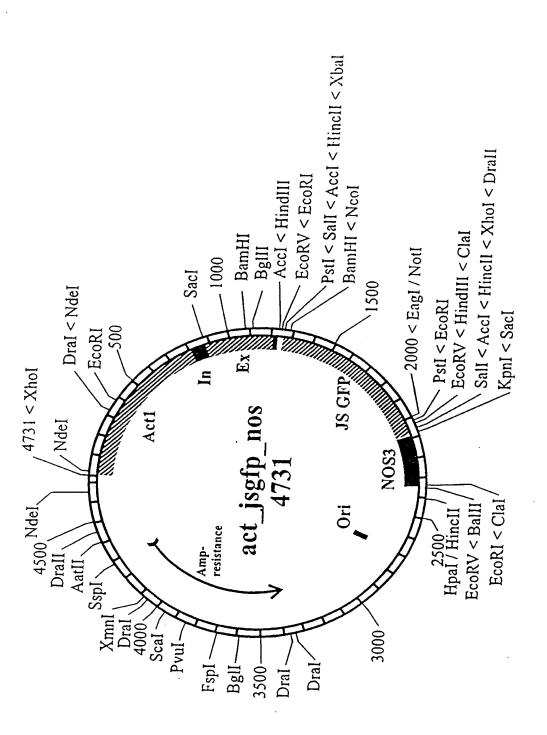
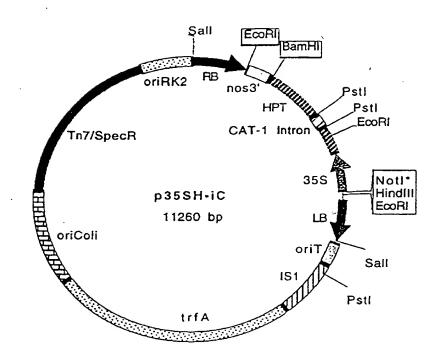


Figure 22E
SUBSTITUTE SHEET (Rule 26) (RO/AU)



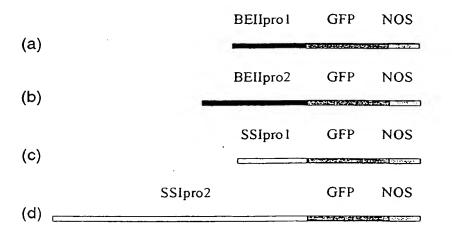


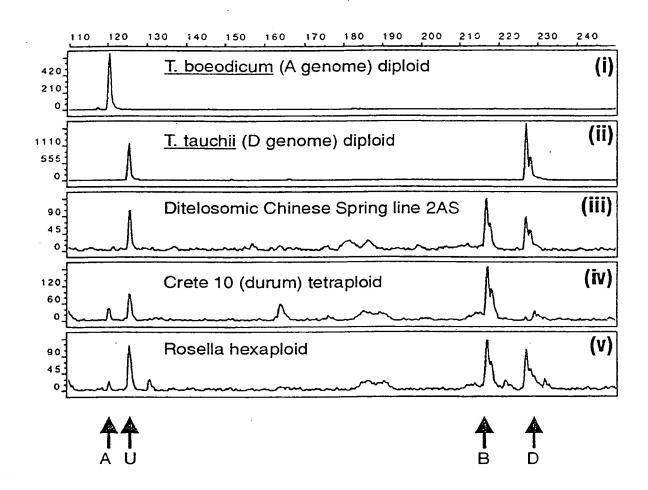
FIGURE 23

Primer	Key	Forward	Forward Primer Sequence
Set		Primer	
1	E01'/E02	WBE2E1F	CGT CGC TGC TCC TCA GGA AG
2	E01/E02	sr854.1180F	CTG GCT GAC TCA ATC ACT ACG
3	E02/E03	WBE2E2F	CGC AAC CTG AAG AAT TAC AG
4	E03/E04	WBE2E3F	ATT TTC GGA GCC ATC TTG AC
5	E04/E05	WBE2E4F	TCG TGG TTA TGA AAA GCT TGG
6	E05/E06	sr913F	ATC ACT TAC CGA GAA TGG G
7	E05/I05	sr913F	ATC ACT TAC CGA GAA TGG G
8	E06/E07	WBE2E6F	ACA ATT GGA ATC CAA ATG CA
9	E07/E08	WBE2E7F	AGC TAT TCC TCA TGG CTC AC
10	E08/E09	WBE2E8F	TGC AGG CTC CAG GTG AAA TA
11	E10/E11	da5.seq	GGC TTG GAT ACA ATG CAG TGC
12	E12/E13	da151.seq	TTG ACG GCT TGA ATG GTT TC
13	E17/E18	WBE2E17F	TTT AGG TGG TGA AGG CTA TCT
14	E18/E19	sr860R	AAT GGA TAG ATT TTC CAA GAG G
15	E19_3′	WBE2-2395F	AGC AGA ACT GCG GTC GTG TA

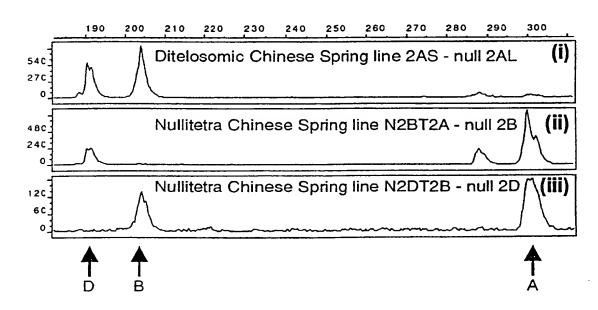
Reverse	Reverse Primer Sequence	Temp	bp
Primer			
WBE2E2R	CAG GAC CTT CCC TGG AGA GG	57.4	401
WSBE9E2R	GGC ACG AGT GTG TGT ACC TGT A	57.7	601
sr866F	TAT CTT CAG GTA TCT ACA GC	49.8	309
WBE2E4R2	ATG CTT CCA ATC CAC CTT CA	-	>450
WBE2E5R	GAG CCC ATT CTC GGT AAG TGA	50.5	234
WBE2E6R	CTG CAT TTG GAT TCC AAT TG	49.9	232
WBE2I5R	CAG TAA GCT AGT TGG TGA ATA	46.6	106
WBE2E7R	GGG AGG AAA ATC TCC CAA AC	51.0	402
sr915F	CCA TTG AAA GGT ATT TCA CC	51.1	203
sr912F	TAA CTT ATT GAC ATA CCG G	48.4	439
WBE2E11R	CTG GAG TTC CAA AAC GGC TAC	51.2	289
WBE2E13R	ATT CTT CAA GCC ACC ATC TC	51.6	244
WBE2E18R	TAT TGT TAT TTC CAG GGG AGA	50.2	258
da23.seq	TGC TGC ATT GCC TGA TCG AA	50.4	~295
WBE2-2634R	AAC ACC CAG GCC CGT CCA TT	57.2	240

# Figure 24

# SBE II Intron 5 primer set - digested with Dde1



# SBE II Intron 10 primer set - digested with Dde1





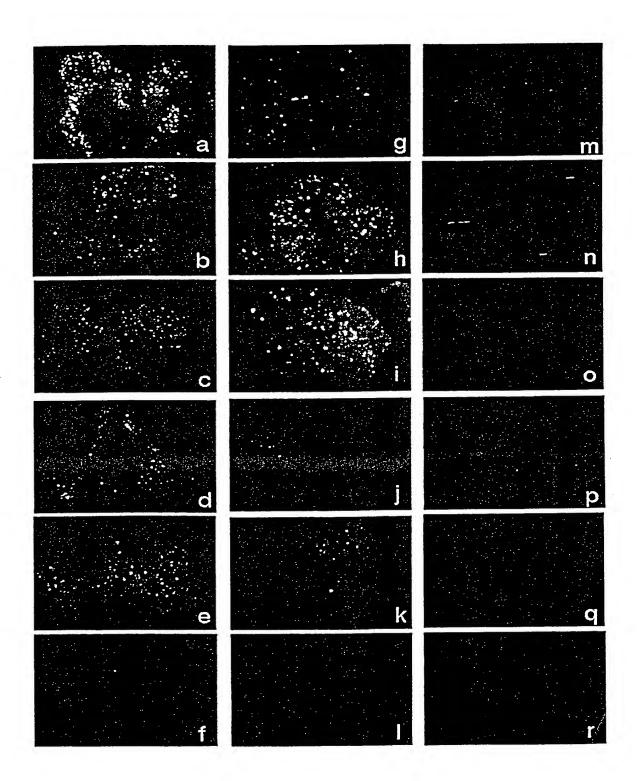


FIGURE 27